Medical implantable devices for the controlled release of anti-TGF-beta1 in the repair of peripheral nerve injuries

Conference or Workshop Item

MEDICAL IMPLANTABLE DEVICES FOR THE CONTROLLED RELEASE OF ANTI-TGFβ1 IN THE REPAIR OF PERIPHERAL NERVE INJURES

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Abstract: The development of novel bioartificial nerve grafts which release soluble therapeutic agents, shows great promises guiding the extension of the injured axons and optimizing and improving the degree and specificity of neural outgrowth. The TGF-β family cytokines are polypeptides involved in pathogenesis of neuropathies during nerve lesion. In particular, studies carried out on TGF-β1 have demonstrated its key-role as a humoral stimulus in scar formation. The use of neutralising antibodies to this pro-fibrotic factor, incorporated and released by medical devices, could be potentially useful to get improved results in nerve repair. The aim of this study was to characterise the uptake and release of antibodies, structurally no different from the anti-TGFβ1 specific ones, by innovative constructs based on the use of biodegradable and biocompatible compounds with which to support and improve peripheral nerve repair.

Introduction

Nerves subjected to mechanical, thermal, chemical, or ischemic insults exhibit a regenerative potential, but, in the absence of surgical reconnection, the recovery of function following a transection or gap injury to a nerve is negligible. The separation between the nerve ends precludes sprouting axons from finding the distal stump and a variety of cellular and humoral events including the inflammatory and scarring response are triggered promoting the growth of connective tissue at the injury site which acts as a physical barrier to neurite elongation. This results in the formation of a neuroma, a dense, irregular tangle of axons which can cause painful sensations [1]. The use of biodegradable medical devices to improve the repair of injured human nerves can be useful to establish a direct, unbroken path between the proximal and distal stumps; to avoid scar tissue invasion into the regenerating environment; to actively promote by contact guidance, the alignment and movement of the elongating neuritis; to avoid tensions, which are known to stimulate the inflammation process and fibrosis, and that can arise in case of extensive deficit, or because of the formation of adhesions between repair and connective tissue; to inhibit the synthesis of pro-fibrotic factors by the release of therapeutic agents [1, 2, 3, 4, 5].

The cytokine Transforming Growth Factor-β1 (TGF-β1), that is expressed both in peripheral and central nervous injuries, plays a pivotal role in the regulation of the immune response and inflammation [6]. It has a multiple role acting as a chemotactic and pro-fibrotic agent enhancing the production of the extracellular matrix components and its neutralisation by specific antibodies (Ab) has shown to be effective to get improved results in the repair of nerve injuries [7, 8].

This study, as a preliminary work, focuses on the *in vitro* characterisation of the absorption and release kinetics of a general monoclonal IgG by medical devices based on natural materials which could be potentially used *in vivo* as controlled delivery systems and nerve guidance grafts [9, 10, 11]. More specifically, constructs made of an inner fibronectin (FN) mat of novel composition and of an outer HYAFF 11 tube (benzyl ester of the hyaluronic acid) were used to carry out the experiments. FN is an extracellular matrix glycoprotein involved in many aspects of wound healing [12] and FN based biomaterials [13, 14, 15] has been used to actively support nerve growth representing the most widely tested forms of contact guidance repair template [16, 17, 18]. Hyaluronan is naturally present at tissue interfaces and joints as a soft tissue lubricant; it is involved in the prevention of mechanical adhesion between connective tissue surfaces [19] and its benzyl ester derivative has already shown to be of efficacy for promoting tissue repair [20, 21].

Materials and Methods

To carry out the experimental work, as a preliminary study, a commercial R-Phycoerythrin labelled Goat Anti-Mouse IgG (whole molecule), produced by Sigma Aldrich Italia (Product number: P9287) was used. This Ab is structurally no different from the specific anti-TGFβ1 one and the molecular weight is equivalent.

The IgG tracing in solution was performed by spectrofluorimetry by means of a Perkin Elmer LS-50B Luminescence Spectrometer (Windows NT based software from Perkin Elmer).

The analyses were performed by means of a quartz square cuvette in order to gain a better signal/noise ratio and to detect even small amounts of antibody with high precision, reliability and reproducibility [22, 23].

The experiments have been carried out at room temperature, in aseptic conditions and in the dark to reduce the fluorochrome molecules photo-bleaching.

In order to inhibit the activity of possible residual proteases of the fibronectin working process, Aprotinin bovine lung (Sigma-Aldrich Italia; product number: A3428) has been used with 300 IU/ml concentration.

Several bioengineered medical devices made of an inner FN mat of novel composition and of an outer HYAFF 11 tube have been tested as controlled delivery systems of monoclonal antibodies.

In order to take into account the possibility that the HYAFF 11 tube outside of the FN mat could affect the drug release kinetics in a different way in constructs of different length, the experiments have been set up in such a way as to characterise the uptake and the release rates of IgG as a function of the device length. The release kinetic has been investigated over a period of 4 moths to evaluate the suitability of the materials to be clinically employed for long term antibody therapies.

In order to reach these aims, 3 guides 1 cm long (type "Long") and 3 guides 0.5 cm long (type "Short") were used. These materials have been dipped in a solution of Ab at the concentration of 5 µg/ml of Phosphate Buffer Saline (PBS)/Aprotinin for 96 hours (passive absorption) and then, each one has been transferred in 400 µl of PBS/Aprotinin to follow the release kinetic.

In order to identify the best wavelengths at which to excite and to detect the emission of fluorescence, the R-Phycoerythrin labelled Goat Anti-Mouse IgG spectra have been characterised in the same physical and chemical conditions used to carry out the experiments. Accordingly to the result gained the analysis were performed exiting the fluorochrome at 470 nm and detecting its emission at 580 nm.

Different standard solutions have been prepared and analysed (5, 3, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, 0.005, 0.001 µgr/ml), and the equation of an appropriate interpolation function has been extrapolated.

After 1, 2, 6, 14, 27 and 120 days, the entire volume of the solution in which the materials were dipped, has been sampled and replaced with other 400 µl of fresh PBS/Aprotinin. The samples have been analysed and the concentration of Ab has been calculated by means of the interpolation. The amount of IgG adsorbed by the constructs has been calculated as the difference between its initial levels inside the solutions in which the materials have been incubated (5 µgr/ml) and those found at the end of the 96 hours of incubation.

Results

The amount of IgG in the solutions in which the materials have been dipped was slightly lowered at the end of the period of incubation.

According to our previous results [24] that showed the ability of similar FN materials to act as a depot of antibody, we assumed the entire amount of IgG lacking in solution was completely absorbed by the FN mats.

As shown in Figure 1 and in Table 1, the tubes long 1 cm absorbed 344.4 nanograms of Ab whereas the shorter ones, 0.5 cm long, absorbed 234.73 nanograms. The total release in 4 months was of 78.85 and 57.64 nanograms respectively for the "long" and "short" tubes; in both cases approximately the 23% of the amount withhold was released. The tubes "long" adsorbed in 96 hours and released in 4 months respectively 1,47 times and 1,37 more Ab than the "short" ones.

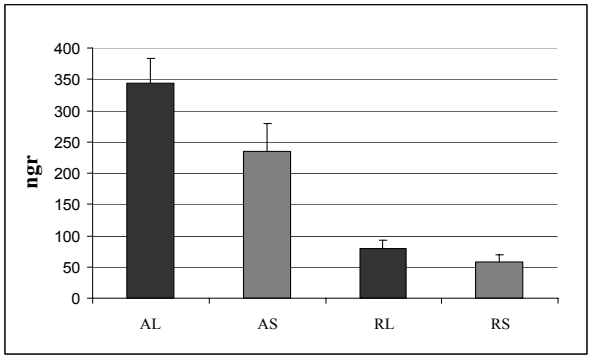

Figure 1: AL: absorption by "Long" tubes; AS: absorption by "Short" tubes; RL: release by "Long " tubes; RS: release by "Short" tubes.

Table 1: amounts of antibody (in nanograms) absorbed and released by the two different sizes of conduits.

	absorption	Release in solution						
		days						total amount
Sampling time	0	1	2	6	14	27	120	
Short	234,73	42,40	4,32	2,65	2,26	2,56	3,45	57,64
Long	344,4	60,74	5,67	3,37	2,30	2,67	4,10	78,85
Long/Short	1.47	1.43	1.31	1.27	1.02	1.04	1.19	1.37

As shown in Figure 2 that shows the Ab released in each interval of time, it is important to note that, for both kind of tubes, the most IgG has been released in the first 24 hours and only really small quantities in the days after.

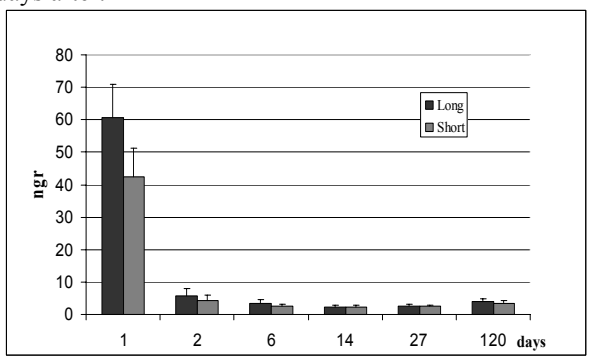

Figure 2: antibody released in each interval of time.

Moreover, as in better evidence in table 1 (Raw: Long/Short), in an initial phase the tubes "Long" released more Ab than the "Short" ones; more specifically in the first 24 hours they delivered 1.43 times more Ab with a decrease of the differences between "long" and "short" tubes mainly after the sixth day.

Discussion

Human anti-TGFβ1 is a therapeutic antibody which blocks the function of human TGF-β1; it has been used in clinical trials for other applications and its biocompatibility is well assessed [25]. In this study, however, a standardised commercial fluorescently labelled IgG preparation was used, since anti-TGFβ1 itself is structurally no different from IgG and the molecular weight is equivalent.

Results from our previous work carried out on HYAFF 11 and different FN compositions [24], stated that only these last ones can act as a depot of Ab releasing it gradually in solution over time. According to this the detected IgG absorption and release dynamics should be then referred exclusively to the Fn mats, even if, it is of importance to take into account the presence of the HYAFF 11 that will have somehow affected the results.

The 1 cm long constructs have not absorbed a double amount of antibody with respect to those 0.5 cm long but less then what would be expected on the basis of a linear relationship (only 1,47 times more). As concerns the release and in particular that of the first 24 hours, the "long" constructs have delivered 1.43 times more Ab than the "short" ones encouraging the guess that the same starting amount of IgG would have been released at the same rate by tubes of different sizes.

The release in the first hours have been rather high compared to that of the following ones; this could be explained assuming that the IgG has not been stored deeply inside the materials, and has therefore been released mostly from the surface of the FN mats and in smaller quantities from the inner layers of these materials. As concern the decrease of the "Long/Short" release ratio over time, it could have been due to a longer IgG release time from the inner regions of the FN mats of the "Long" constructs. Because of the presence of the outer HYAFF 11 tube, the lateral surfaces of the constructs are probably the main if not the only ones subject to the diffusion of the antibody in solution and, as the size of the constructs increases, longer times are probably necessary to have the IgG released through these regions.

On the all, the levels of IgG released in solution have been rather low, mainly after the first 24 hours. In this respect, it is worthwhile to note that the amount of drug inside the constructs can affect the drug release kinetics [1]. The low release rates observed could be then related to the small amounts of antibody spontaneously absorbed by the materials, and to a higher mass of IgG stored inside of the devices could probably correspond a higher concentration of antibody in solution and a prolonged release over time.

Conclusions

The results show a burst of release within the 24 hours with a steep decrease in the following days. It is likely that the physiological levels of anti-TGFβ1 [26] needs higher amounts of Ab to be inactivated but in order to clearly establish if the constructs are suitable or not for an *in vivo* application to induce the anti-fibrosis effect further work would be required.

More specifically, it must be better elucidated in which way the release rates in solution are affected by the amount of Ab inside of the materials; moreover the variability of these dynamics will have to be investigated as a function of the device length at physiological temperature.

In order to reach these aims, instead of carrying out a passive absorption, it will be of importance to directly load different known quantities of Ab inside different sized bioartificial nerve grafts.

Moreover, in order to better understand in which way the Ab is released by the Fn mats and to clarify the role of the HYAFF 11 outer tube in affecting this process, fluorescence microscopy techniques will be used, tracing the signal of the antibody in the thickness of the materials over time. The use of a more stable and smaller Ab fluorescent tag will be taken into account, in order to improve the detectability of the labelled IgG even at lower concentrations, avoiding at the same time artefacts in the release kinetic and underestimation of release. To confirm the results of this preliminary work a specific anti-TGFβ1 will be used, in place of a general IgG; the use of the human anti-TGFβ1 itself will be crucial, in order not to have cross-reactions of the Ab with FN or other materials.

The future expected results will be also useful to set up a mathematical model with which to give a first level estimation of the retention time of the Ab in the bioengineered materials of interest as a function of its initial amount and of the devices length. More specifically, more quantitative, direct measurements will be designed in order to calculate the diffusion coefficient of the human anti-TGFβ1 through the devices. The modelling environment will allow the surgeons who intend to treat peripheral nerve injures with such an Ab to have a reliable estimations of the amount of therapeutic agent to be loaded in the device, taking into account the length of the gap to regenerate and the type of materials used to set up the controlled delivery system.

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